K64 Fentanyl in Blood and Head Hair From Postmortem Cases

Amanda Jenkins, PhD, and Carrie Mazzola, The Office of the Cuyahoga County Coroner, 11001 Cedar Avenue, Cleveland OH 44106; and Christine Moore, PhD, Immunalysis Corporation, 829 Towne Center Drive, Pomona, CA 91767*

The goal of this presentation is to assess the prevalence of fentanyl in blood and corresponding head hair specimens in postmortem cases.

Routine testing methodologies in hair and blood analysis may not include fentanyl. With the use of transdermal delivery systems and potential lacing of street drugs with fentanyl, a need to develop a rapid and sensitive assay for this drug became apparent.

Specimens: Biological Specimens were obtained during the medicolegal death investigation process at The Office of the Cuyahoga County Coroner, Cleveland. Cases were included if there was a history of heroin and/or (transdermal) fentanyl use or fentanyl was detected in any routine assay, such as the basic drug screen.

***Methods* - Blood:** Biological Fluid testing was performed at The Office of the Cuyahoga County Coroner, Cleveland. Blood (heart or femoral) were assayed by solid phase extraction followed by gas chromatography/mass spectrometric analysis in the selected ion monitoring mode. Norfentanyl, fentanyl, alfentanil and sufentanil were included in the assay. Matrix matched calibrators were assayed at 1, 2, 5, 15, and 25 ng/mL with deuterated (d_5) fentanyl and norfentanyl as internal standards. A negative and positive control at 10 ng/mL fentanyl were assayed with each batch. The coefficient of determinations (r^2) were typically >0.99. The linear range of the assay was 1-50 ng/mL. Within (n=13) and between (n=5) day precision for fentanyl at 15 ng/mL was 4.28%CV and 6.52% CV, respectively. Accuracy at 5 ng/mL was 93.2% (n=5).

HAIR: Hair testing was performed at Immunalysis Corporation, Pomona, CA. A screening procedure for the detection of several medica- tions using ELISA included washing 10 mg specimens briefly with acetone and air drying. Following cutting, 0.025M phosphate buffer (1.5 mL) was added. The samples were sonicated at 75°C for three hours, 0.2 mL of supernatant was removed and 0.8 mL of bovine serum albumin (BSA) added to dilute the sample 1:5. A specific aliquot of the extract was used for the ELISA analysis depending on the drug. Presumptively positive samples were re-aliquoted, washed, cut and sonicated in 0.025M-phosphate buffer (pH 2.7; 1.5 mL) for two hours at 75°C with corresponding internal standard. The buffer was removed, and 0.1M sodium phosphate buffer (pH 6.0; 1 mL) added; the samples were subjected to solid-phase extraction. Confirmation was achieved using two techniques. For 2-dimensional GC/MS, the extracts were reconstituted in ethyl acetate (40 µL) and transferred into auto sampler vials. The ions monitored were 250.2 and 151.1 for deuterated (d5) fentanyl; 245.2, 146.1 and 189.1 for fentanyl with a dwell time of 70 ms. The system was operated in electron impact mode. Alternatively, for LC/MS/MS analysis, the instrument was operated in atmospheric pressure chemical ionization positive mode and the collision gas was nitrogen. Two transitions were monitored (337.4 to 188.3; 337.4 to 105.3) and a ratio calculated so as to increase confidence in the result. Both procedures had a limit of quantitation of 10 pg/mg.

Results: 23 cases were identified for inclusion in the study. The majority of decedents were white (83%) and the sexes were evenly divided (52% male). The age range was 30-94 years with a mean±SD of 58.7±18.0, and a median of 51 years. Twelve individuals had a history of transdermal patch use, 9, a history of heroin use, 1 individual both and one with no history. Fentanyl was detected in the blood of 14 cases (n=21) in a concentration range of 1-33 ng/mL (8.78±9.25, 5.50 ng/mL). All cases with a history of patch use were positive for fentanyl (1-33 ng/mL). The corresponding hair specimens screened positive for 12/13 of these cases with fentanyl levels ranging 55-5120 pg/mg.

Only two individuals with a heroin use history were positive in the blood for fentanyl (7, 24 ng/mL). The corresponding hair was positive at 36 and 1295 pg/mg. The hair of one individual with a heroin use history was positive for fentanyl at 31 pg/mg but the corresponding blood was negative. 6-Acetylmorphine, codeine, morphine, ethanol and cocaine metabolites were identified in blood.

Conclusion: Although the highest concentration of fentanyl in hair and blood occurred in cases with the highest patch dose, there did not appear to be a relationship between blood and corresponding hair concentrations or the dose. The data demonstrated that fentanyl is detectable in hair and may be a useful adjunct to routine specimens in postmortem toxicological analysis.

Fentanyl, Blood, Hair

 ** Presenting Author*